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(54) A medicament for the treatment of a fibrotic disorder, and anti-fibrotic composition.

(57) Human interferon is utilised in the manufacture of a medicament for the treatment of a fibrotic disorder. The medicament is administered to raise the total concentration of human interferons in the affected tissue area to about 10 to about 4×10^7 IU of interferon per cubic centimeter. The human interferon used can be naturally derived or recombinant DNA-derived alpha, beta or gamma-interferon. The interferon can be adminstered by any indicated conventional method and in any suitable, pharmaceutically acceptable, compatible vehicle.

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A medicament for the treatment of a fibrotic disorder, and anti-fibrotic composition.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to methods and compositions for treating fibrotic tissue disorders in human patients.

2. Description of the Prior Art

Tissue fibrosis, characterized by excessive deposition of connective tissue components (most notably collagen) is the major pathological feature in various clinical conditions. The collagen deposition can take place in various internal organs, as in pulmonary fibrosis or liver cirrhosis. Skin is also commonly affected by fibrotic processes, and dermal fibrosis is the clinical pathological hallmark of several acquired and heritable cutaneous disorders.

In most cases, fibrosis is a reactive process, and several different factors can apparently modulate the pathways leading to tissue fibrosis. Such factors, largely elaborated by the inflammatory tissue reaction, include the local expansion of fibroblast subpopulations, immune modulation of the synthetic functions of fibroblasts, and altered regulation of various metabolic reactions governing the biosynthesis and degradation of the connective tissue components. Thus, the net accumulation of collagen in fibrosis is a result of imbalance between the factors leading to production and deposition or degradation and removal of collagen.

Although various modalities have been utilized to treat fibrotic diseases and disorders, none of these treatments have been particularly effective because they generally are directed to the symptoms of the disorders but not at the underlying pathology, namely, the imbalance in the metabolic factors regulating production, deposition, degradation and removal of collagen and other connective tissue components. Thus, for example, while topical corticosteroids have been used with some degree of success in treating the early, inflammatory stage of cutaneous keloid formation, such steroid therapy has little or no effect on the later, fibrotic stage when the keloids are actually formed as a result of excess collagen production.

It has been discovered in recent years, however, that activated T-lymphocytes and monocytes/macrophages can effectively modulate several fibroblast functions through the release of soluble macromolecular factors, collectively categorized as lymphokines (for the factors released by lymphocytes) and monokines (for the factors released by monocytes such as macrophages). Among the fibroblast functions modulated by these lymphokines and monokines is the production of fibrosis-forming collagen. See, e.g., Duncan, M.R., et al., J. Invest. Dermatol. , 83:377 (1984). In 1985, we identified gamma-interferon as the lymphokine and beta-interferon as the monokine responsible for inhibition of fibroblast collagen production as well as inhibition of late, but not early, fibroblast proliferation. Duncan, M.R. and Berman, B., J. Exp. Med., 162:516-27 (1985).

In a paper first published in full in 1987, we disclosed our later discovery that a reduced-collagen-producing phenotype in cultured scleroderma fibroblasts persisted after short term exposure to interferons, whether alpha-, beta- or gamma-. Duncan, M.R. and Berman, B., J. Clin. Invest., 79:1318-24 (1987). Notwithstanding that discovery, which was based solely on in vitro data, it was not at all clear whether interferons would be useful in the treatment of fibrotic tissue disorders in vivo, particularly disorders such as cutaneous keloid formation, scleroderma, progressive systemic sclerosis, and the like, where mere restoration of normal fibroblast collagen production levels may prevent further excess collagen deposition, but will not remove or degrade the fibrotic lesions already formed. Moreover, because of the complexity of the interacting metabolic factors relating to connective tissue matrix formation and degradation, the fact that interferons appeared to cause a persistent inhibition of fibroblast collagen production in vitro by no means proved that a similar effect would be observed in vivo.

Hence, notwithstanding the foregoing discoveries, there has been no method disclosed in the prior art for treating fibrotic tissue disorders in humans in a safe and effective manner to inhibit further fibrotic tissue formation and to reduce or remove entirely already-formed fibrotic lesions.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method of treatment of fibrotic tissue disorders in humans which enables both prophylaxis and reversal of such disorders.

It is a further object of the present invention to provide a method of treatment as described above which comprises the administration of a safe and effective pharmacologically active agent to inhibit

excess collagen production and induce the degradation of excessive connective tissue matrix.

In keeping with the foregoing objects and others which will become apparent hereinafter, the invention resides, briefly stated, in the local or systemic administration to a human patient suffering from a fibrotic tissue disorder of an amount of a human interferon sufficient to raise the total concentration of human interferons in the fibrotic tissue area from about 10 to about 4 x 10^7 international units (IU) per cubic centimeter.

As will be further detailed below, the administration of human interferons by suitable methods and in suitable amounts sufficient to raise the concentration in the affected tissue area to the aforementioned concentration range serves the dual purposes of altering the phenotype of excess collagen-producing fibroblasts as well as accelerating the enzymatic degradation of excess connective tissue matrix already deposited. The result, which has been clinically observed, is the inhibition of further fibrotic tissue formation and the removal of existing fibrotic tissue to return the affected area to a normal state.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph showing the variation over time in the lesional area of a keloid treated in accordance with the present invention as specifically detailed in the Example set forth at the end of this specification.

FIG. 2 is a graph showing variation over time in the production of various connective tissue matrix factors by keloid fibroblasts before and after in vivo treatment in accordance with the present invention, expressed as a percentage of normal fibroblast production of those factors.

FIG. 3 is a table comparing the quantities of extracellular collagen derived from cultures of keloid fibroblasts, pre-and post-in vivo treatment, and normal, untreated fibroblasts. The table also compares collagen production of the same cells after in vitro treatment with interferon-alpha$_{2b}$.

FIG. 4 is a table comparing the quantities of glycosaminoglycans derived from the same in vitro cultures of fibroblasts as in FIG. 3.

FIG. 5 is a table comparing the quantities of fibronectin derived from the same in vitro cultures of fibroblasts as in FIG. 3.

FIG. 6 is a table comparing fibroblast proliferation times in the same in vitro cultures of fibroblasts as in FIG. 3.

FIG. 7 is a bar graph illustrating the comparative amounts of collagenase derived from in vitro cultures of keloid fibroblasts pre- and post-in vivo-treatment, and normal, untreated fibroblasts.

FIG. 8 is a table comparing the quantities of collagenase derived from the same in vitro cultures of fibroblasts as in FIG. 3.

DETAILED DESCRIPTION OF THE INVENTION

Whereas we had previously disclosed that human interferons acted as persistent fibroblast deactivators, inhibiting both the growth and collagen production of normal fibroblasts and hypercollagen-producing fibroblasts, we have now discovered that human interferons also increase the collagenase activity of the fibroblasts, either by increasing collagenase production to normal levels in diseased fibroblasts or by removing some yet unknown collagenase inhibiting factor. This discovery led to the development of the method of the present invention, which has been found effective in vivo in treating human fibrotic disorders.

The novel method of the present invention comprises the administration to a human patient suffering from a fibrotic tissue disorder of a sufficient amount of human interferon to raise the initial total concentration of human interferons in the affected tissue area to about 10 to about 4 x 10^7 IU/cc. As used herein, "total concentration of human interferon" refers to the combined concentration, expressed in IU/cc, of all human interferons in the tissue area, whether, alpha-, beta- or gamma-interferons. In the case of dermal fibrosis, for example dermal keloids or scleroderma, the desired tissue concentration level may be achieved by injecting the human interferon intralesionally in sufficient quantity to achieve the necessary concentration, based on the size of the lesion. In the case of systemic fibrosis, intramuscular or intravenous routes of administration can be utilized. In the case of pulmonary fibrosis, the interferon can be incorporated into a solution to be administered intrabronchially by means of an inhalator.

In general, the specific method and route of administration selected is dependent on an assessment of the most efficient and quickest method to achieve the necessary inteferon concentration at the affected site. The method of the invention is not limited to any specific route of administration, and any method or route of administrations known to those skilled in the medical and pharmaceutical arts which achieves the desired tissue concentration of interferon is comprehended by the present invention.

The human interferons utilized in the method of

the present invention may be naturally derived or recombinant DNA-derived human interferon. Alpha (leukocyte), beta (fibroblast) or gamma (immune) interferons, and their subtypes, may be effectively used in the novel treatment methods.

Although all three types of interferon may effectively be used for the purpose of the present invention, in vitro data suggest that alpha and beta interferons, and their subtypes, may have more activity in vivo than gamma-interferon because of their greater effect on increasing fibroblast collagenase activity. Human recombinant DNA-derived $alpha_{2b}$-interferon has been shown particularly effective in vivo.

The interferon composition administered according to the present invention may comprise one or more human interferons, whether of natural origin or recombinant DNA-derived, in any pharmaceutically acceptable vehicle suitable for the preferred route of administration to be utilized in treating a particular condition. Thus, if a parenteral route of administration is required, a suitable injectable solution of human interferons may be prepared by any conventional means known in the pharmaceutical arts. For example, such an injectable solution may compromise sterile isotonic saline, preservatives such as methylparaben or propylparaben, pH adjusters, buffers, and the like. Similarly, any standard vehicle known in the art to be suitable for use in a vehicle for inhalation agents may be utilized to create a vehicle for the interferons to be administered as a nebulized spray from an inhalator, e.g., for treatment of pulmonary fibrosis. Suitable emulsifiers, dispersing agents, wetting agents and the like may also be present.

A wide variety of suitable parenteral and inhalant vehicles are set forth in the standard text entitled "Remington's Pharmaceutical Sciences" - (17ed. 1985). The invention is not limited to any particular vehicle or formulation for the human interferons administered. It is only necessary that the vehicle be compatible with human interferons and one in which the interferons may be easily dissovled or dispersed.

The method of the present invention is useful in treating any fibrotic disorder in humans characterized by excessive fibroblast production of connective tissue matrix, including collagen, fibronectin and glycosaminoglycans (GAG). This includes, among others, the following disorders:

cutaneous keloid formation
progressive systemic sclerosis (PSS)
liver cirrhosis
idiopathic and pharmacologically induced pulmonary fibrosis
chronic graft versus-host disease
scleroderma (local and systemic)
Peyronie's disease
pharmacologically induced fibrosis of the penis
post-cystoscopic urethral stenosis
post-surgical internal adhesions
idiopathic and pharmacologically induced retroperitoneal fibrosis
myelofibrosis

To treat each of the above disorders, it is necessary only to administer a sufficient quantity of one or more human interferons, whether naturally derived for recombinant DNA-derived, to raise the total tissue concentration of human interferon in the affected area to about 10 to about 4×10^7 IU/cc. A more preferred range of tissue concentrations of interferon is from about 10^3 to about 10^7 IU/cc. Administration of the interferons by any appropriate route and in any preferred vehicle may be repeated as often as deemed necessary to achieve the therapeutic goal of restoration of normal fibroblast function and degradation of fibrotic lesions. In each instance, however, the administration of interferon should raise the total tissue concentration of interferons in the affected area to the range specified above.

The dosage amounts of interferon administered to achieve the desired concentration in the affected tissue area will generally be higher when systemic routes of administration are utilized, as opposed to local administration, because upon sytemic administration the interferons will be diluted and metabolized to some degree before reaching the fibrotic site. The determination of dosage amounts of interferon on a case-by-case basis to achieve the desired tissue concentration levels is within the skill of those knowledgable in the medical and pharmaceutical arts.

As set forth in greater detail in the following Example, the method of the present invention was found clinically effective in the treatment of a progressively enlarging cutaneous keloid. After only two intralesional injections of an interferon, the lesional area was reduced by 41%. Moreover, fibroblasts cultured from the keloid prior to and after interferon injection were compared with fibroblasts cultured from the patient's normal skin, revealing that while pre-interferon fibroblasts produced more collagen, more glycosaminoglycans and less collagenase than the patient's own normal fibroblasts, the post-interferon fibroblasts persistently produced normal or sub-normal amounts of collagen and glycosaminoglycans and exhibited normalized levels of collagenase activity. The result of the interferon treatment was, thus, not only cessation of fibrotic tissue production but reduction of the already existing fibrotic lesion through the normalization of collagenase.

The following Example provides a detailed illustration of the method according to the present invention for treating fibrotic disorders in humans.

The Example is not intended, however, to limit or restrict the scope of the invention or the nature of the conditions for which the invention is indicated in any way. Moreover, the Example should not be construed as providing dosage amounts or regimens, routes of administration or specific vehicles for the human interferons which must be utilized exclusively in order to practice the present invention.

EXAMPLE

Patient Treatment

A 55 year old man with a history of post-surgical cutaneous keloid formation developed a keloid in a site on his upper right arm 10 weeks after receiving 200 W/cm^2 as a test dose prior to planned CO_2 laser ablation of his cafe-au-lait macule. The untreated lesion was biopsied under 1% lidocaine local anesthesia before interferon treatment (pre-IFN) and then injected with 1.5 million IU IFN-alpha$_{2b}$ in 0.15 ml of solution using a 30-gauge needle immediately and 5 days later. On the 9th day the keloid was biopsied (post-IFN) as was normal appearing skin in a corresponding anatomical site. The keloid was further injected with 1.5 million IU IFN-alpha$_{2b}$ in 0.15 ml on days 31, 33, 37, 38 and 40. Lesional area was calculated gravimetrically from lesional outlines drawn on plastic.

Interferons

The patient's keloid received intralesional injections (1.5 million IU/injection) of recombinant DNA-derived human IFN-alpha$_{2b}$ (Intron A; Schering Corporation, Kenilworth, N.J.). Recombinant DNA-derived human IFN-alpha$_{2b}$ (SCH 30500) used in the in vitro studies described herein was produced in Escherichia coli and supplied by the Schering Corporation, Kenilworth, N.J. This IFN$_{2b}$ preparation (6.6 x 10^7 IU/ml of phosphate buffered saline; specific activity of 1.2 x 10^8 IU/mg protein) was added directly to fibroblast cultures after dilution to desired concentrations with Dulbecco's modified Eagle's medium (DME) containing 0.1% human serum albumin.

Fibroblast Cultures

Primary fibroblast cultures were initiated from full thickness 2 mm punch biopsies using an explant method. The explants were cultured in 24 well microculture plates (2 cm^2 surface area; Linbro: Flow Labs, McLean VA) in DME containing 25 mM Hepes, 2mM glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin plus 20% heat-activated fetal calf serum (FCS) (Whittaker-MA Bioproducts, Walkersville, MA) at 37° C in 5% CO_2 humidified atmosphere. These primary fibroblast cultures approached confluency after 36 to 42 days of growth and were subsequently trypsinized and subcultured in DME + 20% FCS with subpassage at weekly intervals. Fibroblasts from selected subcultures were then assayed for functional activity, namely, fibroblast growth, collagen production, glycosaminoglycan (GAG) production, fibronectin production and collagenase production.

Clinical Response

As shown in FIG. 1, the keloid injected intralesionally with IFN-alpha$_{2b}$ on days 0 and 4 rapidly reduced in area by 41% by day 9 at which time a post-IFN bopsy of the keloid was performd. In light of the rapid increase in the area of the keloid which peaked on day 31, 5 additional IFN-alpha$_{2b}$ doses were injected intralesionally during the subsequent 9 days. These treatments again resulted in a rapid, persistent reduction in lesional area (46% at day 94). Qualitatively, following each set of injections, the keloid became markedly softer and less raised. The patient experienced transient myalgias, which required no treatment, following the initial set of injections.

In Vitro Production of Matrix Components By Keloidal and Normal Fibroblasts

As summarized in FIG. 2, pre-IFN keloidal fibroblasts in vitro displayed an activated phenotype characterized by persistent hypercollagen and hyper-GAG production compared to the patient's normal fibroblasts. The initial set of IFN-alpha $_{2b}$ injections in vivo resulted in a persistent de-activation of the keloidal fibroblast phenotype, with post-IFN keloidal fibroblasts producing normal or sub-normal amounts of collagen and GAGs. This persisten de-activation was selective, as the normal level of pre-IFN keloidal fibroblast production of fibronectin, another component of connective tissue matrix, remained normal in post-IFN keloidal fibroblasts. As detailed in Tables I and II (FIGS. 3 and 4), the addition of IFN-alpha$_{2b}$ to confluent

cultures of pre-IFN keloidal, post-IFN keloidal or normal fibroblasts resulted in a dose-related reduction in collagen and GAG production. Interestingly, a concentration range of 10^3 - 10^5 IU/ml, which theoretically is attainable locally in vivo, normalized the otherwise persistent hypercollagen and hyper-GAG production by pre-IFN keloidal fibroblasts. As shown in Table III (FIG. 5), in vitro exposure of different passages of any of the 3 fibroblast lines to IFN-alpha$_{2b}$ (10^5 IU/ml) failed to alter their normal level of firbonectin production.

In Vitro Proliferation of Keloidal and Normal Fibroblasts

The observed effects of in vivo IFN-alpha$_{2b}$ on in vitro functions of keloidal fibroblasts were not secondary to an anti-proliferative effect. Confluent non-proliferating cultures were examined, and as shown in Table IV (FIG. 6), in vivo exposure to IFN-alpha$_{2b}$ failed to exert any lasting effect on proliferation, with the doubling time of pre-IFN, post-IFN and normal fibroblasts being virtually identical ($p>0.5$). It is likely that, similar to its effect in vitro (Table IV), exposure to IFN-alpha$_{2b}$ inhibits fibroblast proliferation in vivo, but its continued presence may be required.

In Vitro Elaboration of Collagenase Activity By Keloidal and Normal Fibroblasts

Although our detection of a persistent reduction of collagen and GAG production by keloidal fibroblasts exposed in vivo to IFN-a_{2b} could ultimately result in an antifibrotic effect, it was important to examine the potential catabolic activity of such fibroblasts on pre-formed collagen, especially in light of the rapid clinical response (FIG. 1). As shown in FIG. 7 keloidal fibroblasts elaborate less than 1/3 the normal amount of collagenase activity, which combined with their hyper-collagen and GAG production could result in the development of a keloid. Interestingly, in vivo exposure to IFN-alpha$_{2b}$ normalized the collagenase activity elaborated by keloidal fibroblasts. This in vitro effect was persistent, being detected in early and late cell passages, in the absence of exogenous IFN. As shown in Table V (FIG. 8), in vitro exposure of each of the 3 fibroblast lines to IFN-alpha$_{2b}$ (10^5 IU/ml) increased the level of elaborated collagenase activity.

It has thus been shown that there are provided methods which achieve the various objects of the invention and which are well adapted to meet the conditions of practical use.

As various possible embodiments might be made of the above invention, and as various changes might be made in the embodiments set forth above, it is to be understood that all matters herein described are to be interpreted as illustrative and not in a limiting sense.

What is claimed as new and desired to be protected by Letters Patent is set forth in the following claims.

Claims

1. The use of human interferon in the manufacture of a medicament for the treatment of a fibrotic disorder in a tissue area.

2. The use according to Claim 1 wherein the fibrotic disorder is selected from the group consisting of cutaneous keloid formation, progressive systemic sclerosis, liver cirrhosis, idopathic and pharmacologically induced pulmonary fibrosis, chronic graft-versus-host disease, scleroderma (local and systemic), Peyronie's disease, pharmacologically induced fibrosis of the penis, post-cystoscopic urethral stenosis, post-surgical internal adhesions, myelofibrosis and idiopathic and pharmacologically induced retroperitoneal fibrosis.

3. The use according to Claim 2 wherein said fibrotic disorder is cutaneous keloid formation.

4. The method according to any one of the preceding claims wherein said human interferon is selected from the group consisting of alpha-, beta-, and gamma-interferons and their sub-types.

5. The use according to Claim 4 wherein said interferon is alpha$_{2b}$-interferon.

6. The use according to any one of the preceding claims wherein said human inteferon is naturally derived.

7. The use according to any one of the preceding claims wherein said interferon is recombinant DNA-derived.

8. A method of treatment of a human patient suffering from a fibrotic disorder in a tissue area, involving the use of medicament manufactured in accordance with any one of the preceding claims, wherein the medicament if adminstered in a quantity sufficient to raise the total concentration of human interferons in the tissue area to between about 10 and about 4 x 10^7 international units per cubic centimetre.

9. A method according to Claim 8 wherein said concentration is between about 10^3 and 10^7 international units per cubic centimetre.

10. An anti-fibrotic composition comprising human interferon and a pharmaceutically acceptable carrier.

11. A composition according to Claim 10 comprising 1.5 million international units of human interferon in 0.15 ml of solution.

12. A composition according to one of Claims 10 and 11 for adminstering in interlesional, intramuscular, intravenous or intrabronchial manner.

13. A composition according to Claim 12 wherein said pharmaceutically acceptable carrier comprises sterile isotonic saline, preservatives and pH adjusters.

14. A composition according to one of Claims 10 and 11 wherein said carrier comprises a pharmaceutically acceptable inhalant vehicle comprising emulsifiers, dispersing agents and wetting agents.

15. Any novel feature or novel combination of features herein described and/or as shown in the accompanying drawings.

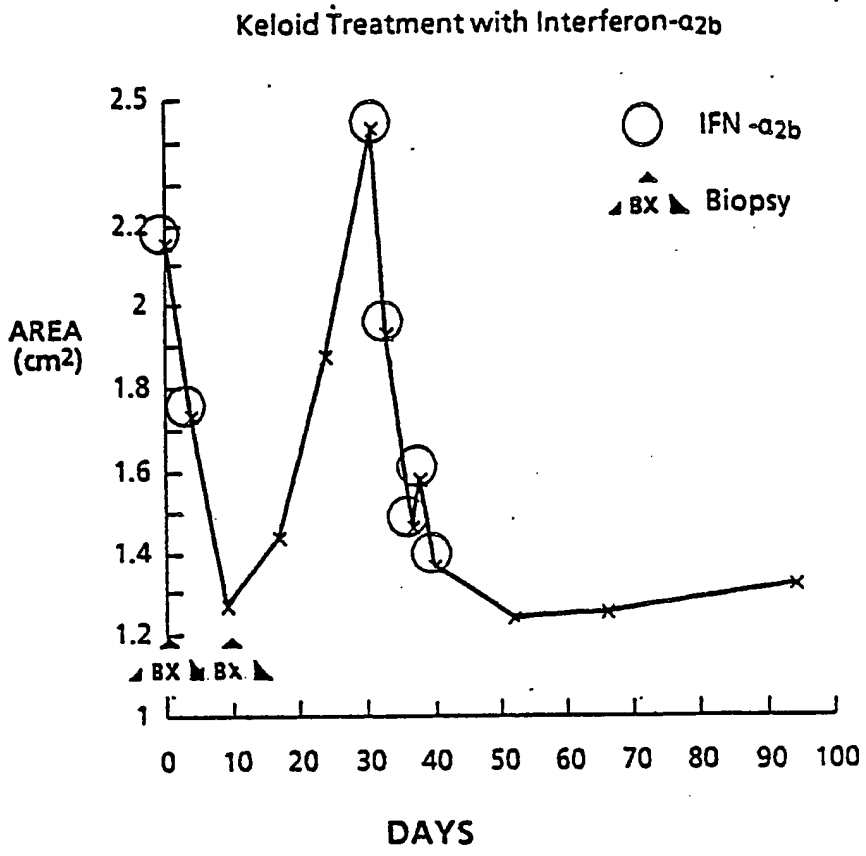
Keloid Treatment with Interferon-α2b
IFN -α2b
BX Biopsy
AREA (cm2)
2.5
2.2
2
1.8
1.6
1.4
1.2
1
BX BX
0 10 20 30 40 50 60 70 80 90 100
DAYS

FIG. 1

In vitro production of matrix components by PRE and POST in vivo IFN-treated keloid fibroblasts

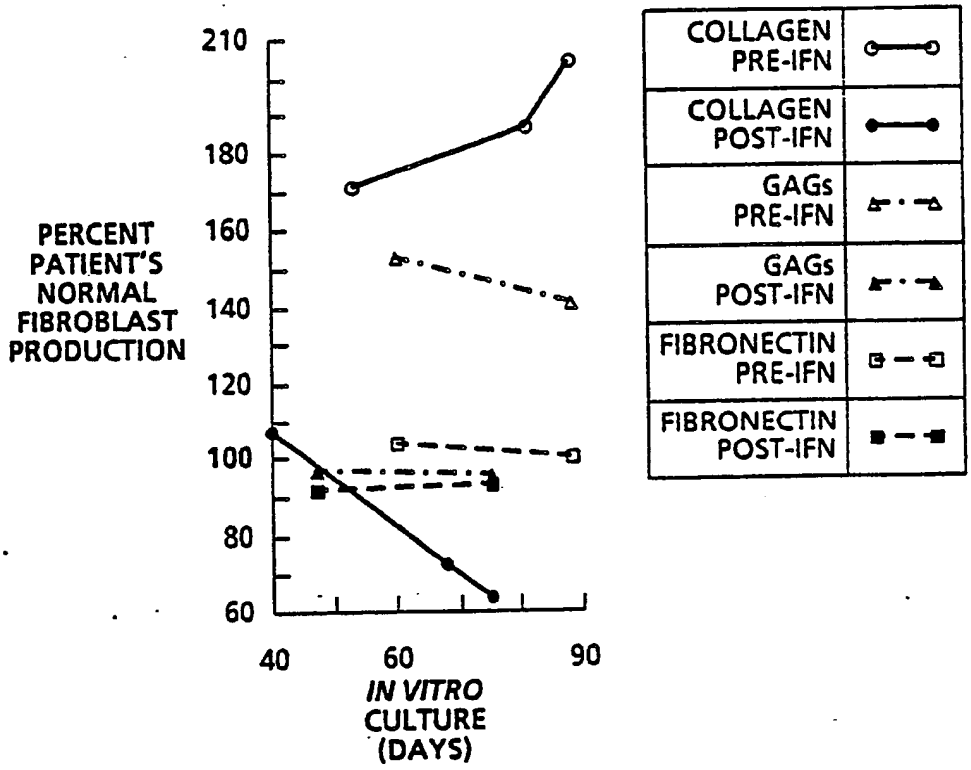

FIG. 2

FIG. 3

FIBROBLAST SOURCE	PASSAGE	^{3}H-COLLAGEN (DPM / 10^3 CELLS ± SD) *IN VITRO*			
		UNTREATED	IFN-α_{2b} 10^5 U/ml	IFN-α_{2b} 10^3 U/ml	IFN-α_{2b} 10 U/ml
KELOID PRE - *IN VIVO* IFN-	2	96 ± 7	48 ± 5	64 ± 4	89 ± 15
KELOID POST - *IN VIVO* IFN-	1	59 ± 5	34 ± 2	44 ± 3	51 ± 1
NORMAL SKIN UNTREATED	1	56 ± 5	23 ± 5	33 ± 1	49 ± 1
KELOID PRE - *IN VIVO* IFN-	6	93 ± 14	55 ± 2	NT	NT
KELOID POST - *IN VIVO* IFN-	5	36 ± 3	25 ± 5	NT	NT
NORMAL SKIN UNTREATED	5	50 ± 9	28 ± 1	NT	NT
KELOID PRE - *IN VIVO* IFN-	7	107 ± 6	43 ± 4	NT	NT
KELOID POST - *IN VIVO* IFN-	6	33 ± 6	24 ± 4	NT	NT
NORMAL SKIN UNTREATED	6	52 ± 6	29 ± 1	NT	NT

Table I. Effect of *in vivo* and *in vitro* Treatment with Interferon-α_{2b} on Collagen Production by Dermal Fibroblasts

FIBROBLAST		^{3}H-GLYCOSAMINOGLYCAN (DPM / 10^3 CELLS ± SD) *IN VITRO*			
SOURCE	PASSAGE	UNTREATED	IFN-α_{2b} 10^5 U/ml	IFN-α_{2b} 10^3 U/ml	IFN-α_{2b} 10 U/ml
KELOID PRE - *IN VIVO* IFN-	3	122 ± 15	56 ± 3	83 ± 5	120 ± 13
KELOID POST - *IN VIVO* IFN-	2	78 ± 9	71 ± 3	69 ± 1	83 ± 13
NORMAL SKIN UNTREATED	2	80 ± 6	47 ± 2	59 ± 1	78 ± 6
KELOID PRE - *IN VIVO* IFN-	7	92 ± 1	61 ± 3	NT	NT
KELOID POST - *IN VIVO* IFN-	6	62 ± 7	58 ± 5	NT	NT
NORMAL SKIN UNTREATED	6	65 ± 3	47 ± 5	NT	NT

Table II. Effect of *in vivo* and *in vitro* Treatment with Interferon-α_{2b} on Glycosaminoglycan Production by Dermal Fibroblasts

FIG. 4

FIBROBLAST		FIBRONECTIN (ug / 10^5 CELLS ± SD) *IN VITRO*	
SOURCE	PASSAGE	UNTREATED	IFN-α_{2b} 10^5 U/ml
KELOID PRE - *IN VIVO* IFN-	3	2.82 ± .01	2.91 ± .04
KELOID POST - *IN VIVO* IFN-	2	2.48 ± .15	2.41 ± .03
NORMAL SKIN UNTREATED	2	2.71 ± .04	2.67 ± .02
KELOID PRE - *IN VIVO* IFN-	7	2.92 ± .02	3.16 ± .03
KELOID POST - *IN VIVO* IFN-	6	2.70 ± .04	2.78 ± .03
NORMAL SKIN UNTREATED	6	2.92 ± .05	3.06 ± .04

Table III. Effect of *in vivo* and *in vitro* Treatment with Interferon-α_{2b} on Fibronectin Production by Dermal Fibroblasts

FIG. 5

FIBROBLAST		CELL NUMBER (X10^{-3} / 2 cm^2 ± SD) *IN VITRO*			
SOURCE	PASSAGE	UNTREATED (DOUBLING TIME IN HRS)	IFN-α_{2b} 10^5 U/ml	IFN-α_{2b} 10^3 U/ml	IFN-α_{2b} 10 U/ml
KELOID PRE - *IN VIVO* IFN-	3	51.8 ± 5.2 (42.6)	19.7 ± 3.3	34.3 ± 5.7	55.0 ± 6.8
KELOID POST - *IN VIVO* IFN-	2	49.0 ± 6.4 (43.1)	22.2 ± 3.6	27.5 ± 3.3	50.0 ± 7.1
NORMAL SKIN UNTREATED	2	46.8 ± 4.8 (44.2)	14.7 ± 2.7	21.9 ± 3.4	49.6 ± 7.2

Table IV. Effect of *in vivo* and *in vitro* Treatment with Interferon-α_{2b} on Dermal Fibroblast Proliferation

FIG. 6

EFFECT OF *IN VIVO* INTERFERON-α_{2b} TREATMENT ON COLLAGENASE PRODUCTION BY KELOIDAL FIBROBLASTS *IN VITRO*

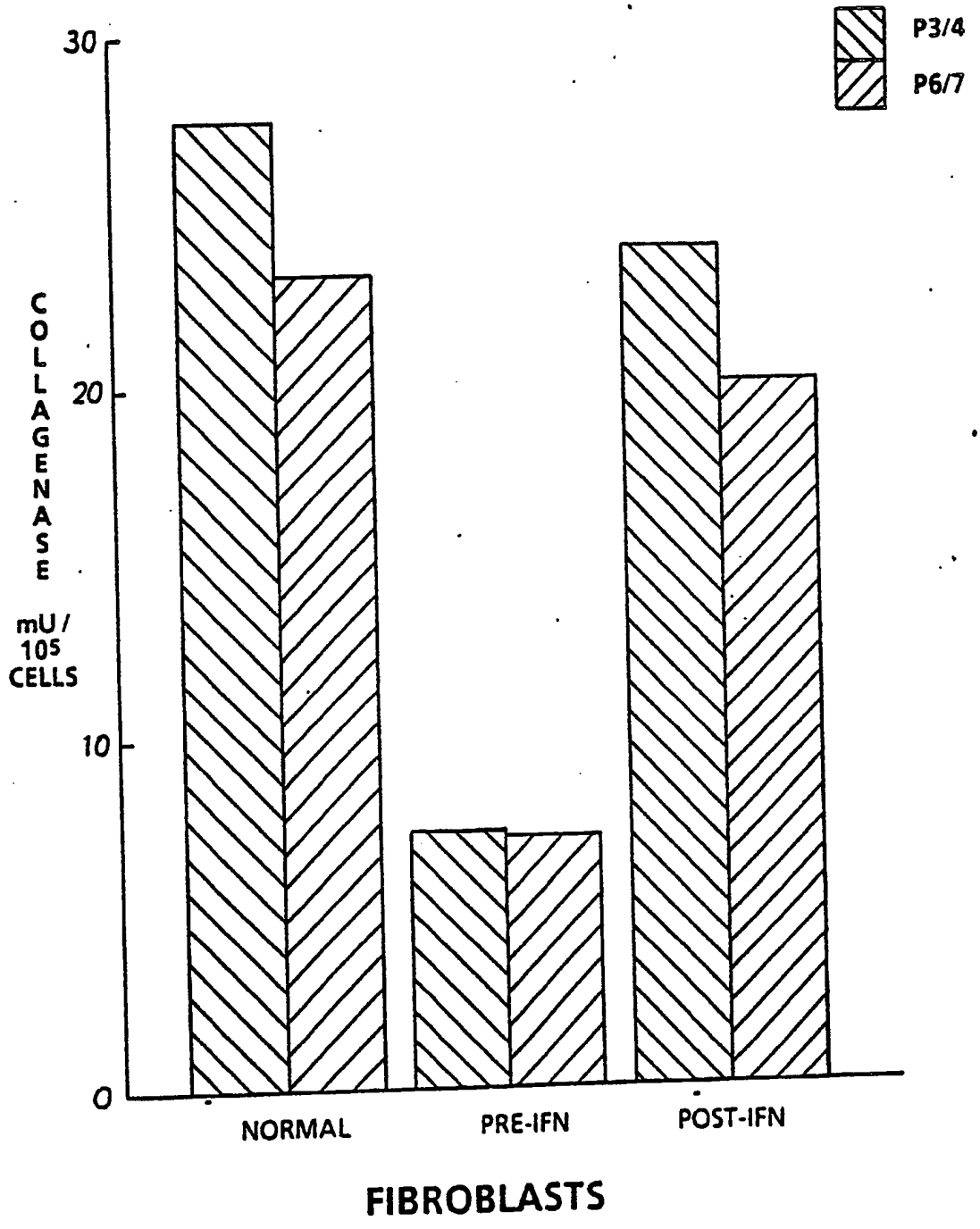

FIG. 7

FIBROBLAST		COLLAGENASE (mU / 10^5 CELLS ± SD) *IN VITRO*		
SOURCE	PASSAGE	UNTREATED	PERCENT NORMAL	IFN-α_{2b} 10^5 U/ml
KELOID PRE - *IN VIVO* IFN-	4	7.4 ± 0.6	26.7	13.1 ± 1.2
KELOID POST - *IN VIVO* IFN-	3	23.9 ± 1.8	96.5	31.6 ± 3.1
NORMAL SKIN UNTREATED	3	27.6 ± 2.4	100.0	37.3 ± 3.4
KELOID PRE - *IN VIVO* IFN-	7	7.2 ± 0.9	31.0	11.6 ± 1.0
KELOID POST - *IN VIVO* IFN-	6	20.1 ± 2.2	86.0	24.3 ± 2.4
NORMAL SKIN UNTREATED	6	23.2 ± 1.8	100.0	36.1 ± 3.9

Table V. Effect of *in vivo* and *in vitro* Treatment with Interferon-α_{2b} on Collagenase Production by Dermal Fibroblasts

FIG. 8

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application number
EP 89 30 0476

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	BIOLOGICAL ABSTRACTS, vol. 85, 1988, abstract 7776 C. CARLO-STELLA et al.: "Effects of recombinant alpha and lambda interferons on the in vitro growth of circulating hematopoletic progenitor cells (CFU-GEMM, CFU-Mk, BFU-E, and CFU-GM) from patients with myelefibresis with myeloid metaplasis" * Abstract * --	1-7, 10-15	A 61 K 45/02
X	BIOLOGICAL ABSTRACT, vol. 84, 1987, abstract 1086 R.D. GRANSTEIN et al.: "Gamma-interferon inhibits collagen synthesis in vive in the mouse" * Abstract * -- ./.	1-4, 6-7, 10-15	TECHNICAL FIELDS SEARCHED (Int. Cl.4) A 61 K

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.

Claims searched completely: 1-7,10-15

Claims searched incompletely:

Claims not searched: 8,9

Reason for the limitation of the search:

Method for treatment of the human or animal body by surgery or therapy (See art. 52(4) of the European Patent Convention)

Place of search	Date of completion of the search	Examiner
The Hague	26-04-1989	FERNANDEZ BRANAS

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
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T : theory or principle underlying the invention
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& : member of the same patent family, corresponding document

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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	BIOLOGICAL ABSTRACTS, vol. 84, 1987, abstract 28220 R.J. SHARPE et al. "Endogenous gamma interferon production may protect against hepatic cirrhesis and administration of exegenous gamma interferon may protect individuals prome to cirrhesis" * Abstract *	1-4, 6-7, 10-15	
X,D	CHEMICAL ABSTRACTS, vol. 107, no. 1, July 6, 1987, page 519 abstract 5512f M.R. DUNCAN et al.: "Persistence of a reduced-collagen-producing phenotype in culted scleroderma fibroblasts after short-term exposure to interferons" & J. CLIN. INVEST. 1987, 79(5), 1318-24 * Abstract *	1-7, 10-15	TECHNICAL FIELDS SEARCHED (Int Cl.4)
X	THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol.262, no. 27, pages 13348-13351 M.J. CZAJA et al.: "Differential effects of lambda-interferon on collagen en fibronectin gene expression" * Front page, abstract and introduction; pages 13349-13350; "Discussion" *	1-7, 10-15	
X	EP-A-0 180 737 (BIOFERON) * Page 1, claim 11 *	1-4, 6-7, 10-15	